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Study on the Efficacy of Vitamin C Lotion on Skin: Permeable and Anti-Aging

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Abstract

Vitamin C (ascorbic acid) plays an important role in maintaining skin health, and topical vitamin C supplementation can counteract oxidative stress induced by UVA, due to excellent reducibility. To clarify the efficacy of vitamin C on the skin in the carrier of lotion, we studied its permeability, irritation and anti-aging effect *in vitro* and *in vivo*, using Franz cell system, cell model and clinical test. The permeability test showed that vitamin C with 10%, 15%, 20% and 25% mass ratio could effectively penetrate skin. The 20% of vitamin C lotion (VCL-20%) had the highest efficiency of transdermal penetration and the diffusion percentage reached 84.707% after 24 h. Besides, the permeation quantity of VCL-20% was 1.43 times that of the control group. Irritation test showed that the cytotoxicity of vitamin C lotion was low. And no allergic reaction happened in the occlusive patch test. Compared with the control group, using vitamin C lotion for 28 days could significantly improve subjects' skin gloss of 10.53% and improve skin color, enhance facial skin elasticity and tightness of 9.20% and reduce wrinkle area of 12.27% ($p < 0.05$).

Keywords

Vitamin C Lotion, Efficacy, Transdermal Penetration, Skin Whitening, Anti-Aging

1. Introduction

The eternal pursuit of beauty has created the development and prosperity of the cosmetics industry. With the development of the social economy and living standards, more and more consumers have begun to pay attention to anti-aging skincare, to maintain a state of younger and more competitive. Many active ingredients have anti-aging efficacy, such as Retinol, Pro-Xylane and Polyphenols. They can be roughly divided into three categories according to their source: plant components, synthetic components and fermentation components. The

synthetic components are favored by consumers with high purity, single component and clear mechanism, for example vitamin A (retinol), vitamin B family and vitamin C (ascorbic acid).

Vitamin C has been used in food and medicine for many years, mainly due to its oxidation resistance. In addition, vitamin C plays an indispensable role in collagen synthesis, which means that it also has the effect of delaying aging. However, the water solubility of vitamin C leads to its poor percutaneous permeability, which also greatly limits its application. In order to dispel consumers' doubts about the permeability of vitamin C, scientists confirmed that the permeability of vitamin C can be enhanced by increasing its concentration. Nevertheless, when vitamin C is used in water-based lotion or emulsion, it is difficult to avoid the problem of oxidative degradation even though a polyol protection strategy has been adopted.

To improve the oxidation stability of vitamin C, a discrete vitamin C lotion (VCL) has been developed in which aqueous phase and powder are separated. Vitamin C is placed in a sealed package as the powder phase, and the lotion is placed in another package tube as the aqueous phase. Above two phases need to be mixed in the palm of hand before use. To further verify the efficacy of vitamin C in the vehicle of lotion, this study will explore the VCL's transdermal efficiency, safety and functional properties through the method of Franz cell system, cell model, occlusive patch test and clinic test.

2. Materials and Methods

2.1. Reagents and Instruments

MTT (3-(4,5)-dimethylthiahiazo (-z-y1)-3,5-di-phenytetrazoliumromide), sodium dodecyl sulfate, fetal bovine serum, pH 7.4 phosphate buffer, potassium ascorbate standard, metaphosphate, etc. were purchased from Solarbio Technology Co., Ltd. MEM (minimum essential medium) was purchased from Thermo Fisher Scientific.

Transdermal diffusion meter (PermeGear, USA); Kq3200e ultrasonic cleaner (Kunshan Shumei); Analytical balance (Mettle-Toledo); Carbon dioxide incubator (Shanghai Santeng); Inverted phase contrast microscope (Leica); High performance liquid chromatograph, centrifuge and microplate reader (Thermo Fisher, USA); Cutometer (Courage + Khazaka, Germany); Skin detector VISIA 7 (Canfield, USA); Image Pro Plus image analysis software (Media Cybernetics); Patch applicator (Finn Chambers, Finland).

2.2. Sample Preparation

Test sample of vitamin C lotion was GALENIC GALENICEUTICALS No.1 BRIGHTENING RADIANCE ENERGY CONCENTRATED CARE (Galenic N˚1), which contains the powder phase and the lotion phase. Table 1 shows the whole formula ingredients. Under normal use, the concentration of vitamin C in the GALENIC N˚1 lotion is 20%. Before each test, the above two phases need to be mixed at a certain mass ratio of vitamin C for testing purposes.

Table 1. The formula ingredients list of vitamin C lotion (Galenic N˚1).

Phase	Ingredients
Powder	Vitamin C
Lotion	De-water
	EDTA-2Na
	Glycerol
	HYDROXYETHYL ACRYLATE/SODIUM ACRYLOYLDIMETHYL TAURATE COPOLYMER
	HYDROGENATED STARCH HYDROLYSATE
	METHYL GLUCETH-20
	POLYSORBATE 60
	DIMETHICONE
	ISOHEXADECANE
	SORBITAN ISOSTEARATE
	SODIUM HYALURONATE
	1,2-HEXANEDIOL
	FRAGRANCE

Besides, the OBAGI C20 L-vitamin C essence (Obagi-20%) was purchased from the flag ship store of OBAGI on TMALL as the control group.

2.3. Franz Diffusion Cell Assay

The experiments were conducted using pig skin in vertical Franz cells [1], whose volume of the acceptor compartment is 8.5 ml. The area of skin effective diffusion was 3.14 cm^2. The receptor chamber was filled with 7.0 mL of receptor fluid (phosphate buffer). The skin (1-month-old suckling pig back skin) was fixed between the supply chamber and the r Receptor chamber. Add 1.5 ml buffer to the receiving chamber through the sampler to exhaust the air. Sink conditions were maintained through all the experiments. A volume of 300 μL of test products was applied onto the skin under a finite regimen administration. After 24 h, the skin surface was washed thoroughly and stratum corneum removed. Remaining epidermis, dermis and receptor fluid were collected. Following an extraction protocol with phosphate buffer, compound levels were quantified by HPLC.

The experiments were conducted in triplicate, carried out at 32˚C and 300 rpm for 24 h. Samples were evaluated at different time points. All samples of the test system were analyzed and recovery of compound versus applied dose was determined. Permeation values were calculated as the sum of compounds present in receptor fluid.

Analytical conditions: The chromatographic separations were performed in a Waters Acquity UPLC BEH C18 (5 μm, 4.6 × 150 mm) column at 30˚C. The sampler volume was 1 μL. The mobile phases consisted of water containing 0.1%

of metaphosphoric acid and methanol (volume ratio: 96:4) at a flow rate of 1 mL/min. The detection wavelength was 243 nm.

Cumulative permeability (Q):

$$Q = C_n \times V_0 + \sum C_i \times V_i \left(i = 1 \cdots n-1\right). \quad (1)$$

where: C_n: The concentration of samples at each collection time point, n. C_i: The concentration of samples at each collection time point i, $i = 1 \ldots n - 1$; V_i: The volume of each sample collection was 2 mL in this study; V_0: The volume of receptor chamber was 8.5 mL in this study.

Diffusion percentage (P):

$$P = \frac{Q}{P_0} \times 100\%. \quad (2)$$

where: Q: Total contents of sample within receptor fluid; P_0: The total dosage of sample.

The permeation coefficient (K_p): Regression equations were performed with cumulative permeation quantity and permeation time were used as variables. And the slope of the equation was the permeation coefficient (K_p).

Rate of recovery (R):

$$R = \frac{Q + W + N}{\text{Total contents}} \times 100\%. \quad (3)$$

where, Q: Total contents of sample in receptor liquid; W: Total contents of sample recovered from the pigskin surface and donor chamber; M: Total contents of sample recovered from pigskin; N: Total contents of sample recovered from pigskin.

Standard curves of vitamin C was $y = 3410460x - 26107.9$, $r^2 = 0.99987$.

2.4. Irritation Test *in Vitro*

Corneal epithelial cells (Fenghui biology) were cultured in Medium. Upon reaching 80% - 90% confluence, cells were seeded into 96-well plates, each treated with 200 μL complete medium. Subsequently, the cell cultures were incubated for 48 h (37˚C, 90% RH, 5% CO_2), and then treated with test products for 30s. Selection of non-cytotoxic concentrations of vitamin C lotion was based on previous results of cytotoxicity assays (data not shown), using MTT assay. Before use, MTT needs to be prepared into a working solution with a concentration of 5 mg/ml. After stimulus processing, cell viability was calculated by a fluorescence microplate reader (Multiskan FC, Thermo Fisher Scientific) at 570 nm.

$$\text{Cell viability}\left(\%\right) = \frac{A_s}{A_b} \times 100\%. \quad (4)$$

where: A_s is the absorbance of sample; A_b is the absorbance of blank control group.

2.5. Clinical Occlusive Patch Test

The tests were conducted in SGS-CSTC Standards Technical Services Co., Ltd. Guangzhou Branch.

Study participants: In this study, 34 healthy women with age of 24 - 58 years participated. None of them has known dermatological diseases or allergic history [2] [3]. All participants were informed about the possible adverse reactions of the study and included after written informed consent. They were allowed to remove the patches if severe irritancy develops in any of the subjects. All participants completed the study. Participants were instructed not to use any skin care products like moisturizers on the test sites 1 day before the study.

This monocentric study was carried out in SGS (Sciete Generale de Surveillance S.A.). Tests were carried out on the inner forearms of healthy adults. Assessments for objective and subjective methods were performed at baseline and after 24 h. The evaluations performed were of visual assessment, for example, redness, scaling, following the exposure period. Before any measurements, all subjects had to rest in Cosmetic Lab, under constant environmental conditions of 22˚C ± 2˚C and 50% ± 5% relative humidity, for at least 30 min.

The area of the inner forearm specified for the test was shaved 4 h before baseline assessment and before application of any test formulation. A small amount of each test product was applied, covering it with suitable closed patch applicator, fixed with adhesive tape. After 0.5 h, 24 h and 48 h, the patches were removed, and the skin was gently cleansed, visual measurements were performed after the indentation disappears. Grading for COLIPA visual scoring method (CVSM) is shown in **Table 2** [4].

2.6. Efficacy Test *in Vitro*

Mice melanocytes (Fenghui biology) were cultured in Medium. Upon reaching 80% - 90% confluence, cells were seeded into 6-well plates, each treated with 3mL complete culture medium. Subsequently, the cell cultures were incubated for 48 h (37˚C, 90% RH, 5% CO_2), and then treated with test substances. Selection of non-cytotoxic concentrations of vitamin C lotion was based on previous results of cytotoxicity assays (data not shown), using MTT assay. After 48 h of incubation, cell lysates were collected for quantification of melanin and tyrosinase inhibitory activity [5].

Quantification of melanin: The precipitate was removed from the cell lysate and the supernatant was prepared by centrifugation. Melanin concentration was measured by a fluorescence microplate reader (Multiskan FC, Thermo Fisher Scientific) at 475 nm and compared to a curve with blank control.

Table 2. Assessment of reaction for irritant patch test.

Grading	Description of skin response
0	No visible reaction
1	Doubtful erythema; Mild erythema
2	Moderate to intense erythema
3	Intense erythema with edema
4	Intense erythema with edema and vesicle

Relative melanin content (*RM*):

$$RM\left(\%\right)=\frac{A_s}{A_b}\times 100\%\,. \tag{5}$$

where: A_s is the absorbance of sample; A_b is the absorbance of blank control group.

Tyrosinase activity inhibitory [6]: Forty microliters of L-dopa (5.0 mM, Sigma-Aldrich) were mixed with 80 μl of phosphate buffer (0.1 M, pH 6.8) in a 96-well microtiter plate and incubated for 10 min at 37˚C. Forty microliters of vitamin C lotion were added to the 96-well plate in serial dilutions. Then 40 μl of tyrosinase (250 U/ml, dissolved in phosphate buffered saline [PBS] media; Sigma-Aldrich) was added to each well on the plate, and the absorbance characteristics of the resulting mixture were measured at 475 nm at 10 min. The anti-tyrosinase activity was expressed as % inhibition of the enzyme tyrosinase.

Tyrosinase activity inhibitory (*TA*):

$$TA\left(\%\right)=\frac{A_b-A_s}{A_b}\times 100\%. \tag{6}$$

where: A_s is the absorbance of sample; A_b is the absorbance of blank control group.

2.7. Efficacy Test *in Vivo*

Subjects selection

Participants for this study were recruited as subjects from SGS and asked to participate, and were informed about the study and signed a consent form. 41 Chinese adult women with healthy skin but visible signs of skin aging who met the inclusion and exclusion criteria were enrolled in this prospective study. The main inclusion criteria were visible signs of loose and gloomy skin, and fine lines at the corners of the eyes. Exclusion criteria were: 1) skin diseases on the face that may affect the judgment of the test results; 2) Highly allergic constitution; 3) Have a history of cosmetics allergy; 4) Women who are pregnant, breastfeeding or intend to become pregnant during the test; 5) Severe impairment of heart, liver and kidney function and severe low immune function.

The total duration of the study was 4 weeks and a total of 4 appointments. Subjects were instructed not to wash their face at least 6 hours before the first appointment and should not apply any skin care product at least 12 hours prior to the study visit. In addition, no make-up, powder or rouge was allowed to be used. A dermatological assessment of the skin was performed as baseline measurement on day 0 of the test.

After this, the test product was used once every three days for 4 weeks on half of the face, while the other face without the test product was used as blank control. During the test, subjects need to use auxiliary samples (sunscreen) all over their face during the day.

Measurements and Assessments

After 1 day, after 1 week and after 4 weeks, a dermatological assessment was done. Following an acclimatization period of 30 minutes, all measurements were

done under standardized room conditions (20˚C and 50% relative humidity) of 12 hours after the last application of the test product.

The following measurements and skin assessments were performed: The skin elasticity F3/F4 value and skin firmness parameter F4 value of subjects' cheeks were measured by cutometers. VISIA-system 7 was used to collect the image of the subject's cheek skin and analyze the skin color with image pro plus image analysis software. Besides, the canthus wrinkle area and skin wrinkle SA value of subjects were measured with Canfield sci skin rapid optical imaging system PRIMOS (Canfield Scientific Inc., Parsippany, New Jersey, USA).

2.8. Statistical Analysis

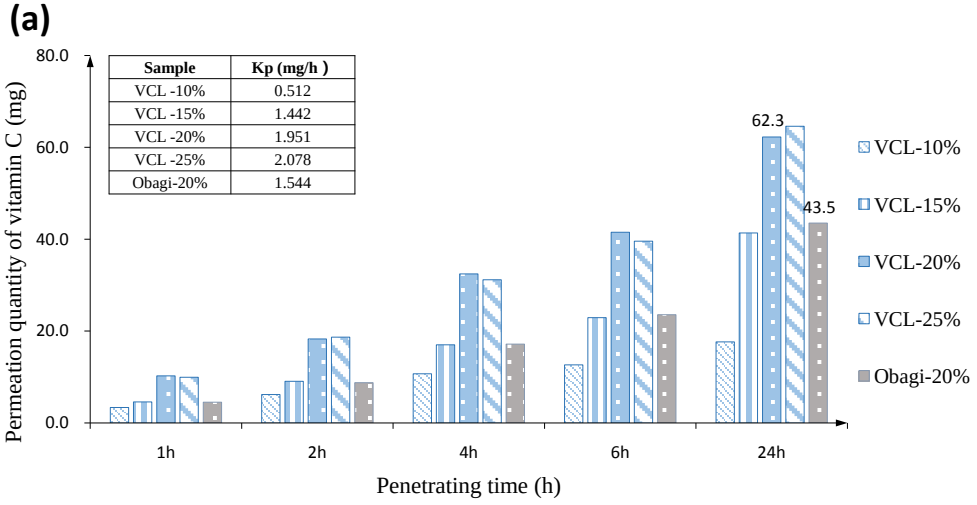
SPSS analysis software was used to test the normal distribution and homogeneity of variance of each parameter before statistical analysis. T-test or rank sum test were used to compare the measured values and basic values at different time points, and the significance was analyzed. A conventional significance level determination of (*) $p < 0.05$ was used as a basis for the overall evaluation.

3. Results

3.1. Permeability of Vitamin C Lotion

This test aimed to evaluate and compare, using the methodology of Franz diffusion cells [7], vitamin C of four concentrations releasing profiles in carrier of lotion. Vitamin C samples of four concentrations were prepared by adjusting the amount of vitamin C in lotion formulation. **Figure 1** shows the *in vitro* transdermal penetration results of vitamin C within 24 h.

The permeation quantity represented a marked dependence on two factors: concentration of vitamin C in lotion formulation and penetrating time, as shown in **Figure 1(a)**. The top two with the highest permeation quantity were obtained for 20% and 25% vitamin C at 24 h, up to 62.3 mg and 64.6 mg, respectively. Besides, the permeation quantity of VCL-20% was 1.43 times that of the control group (Obagi-20%).



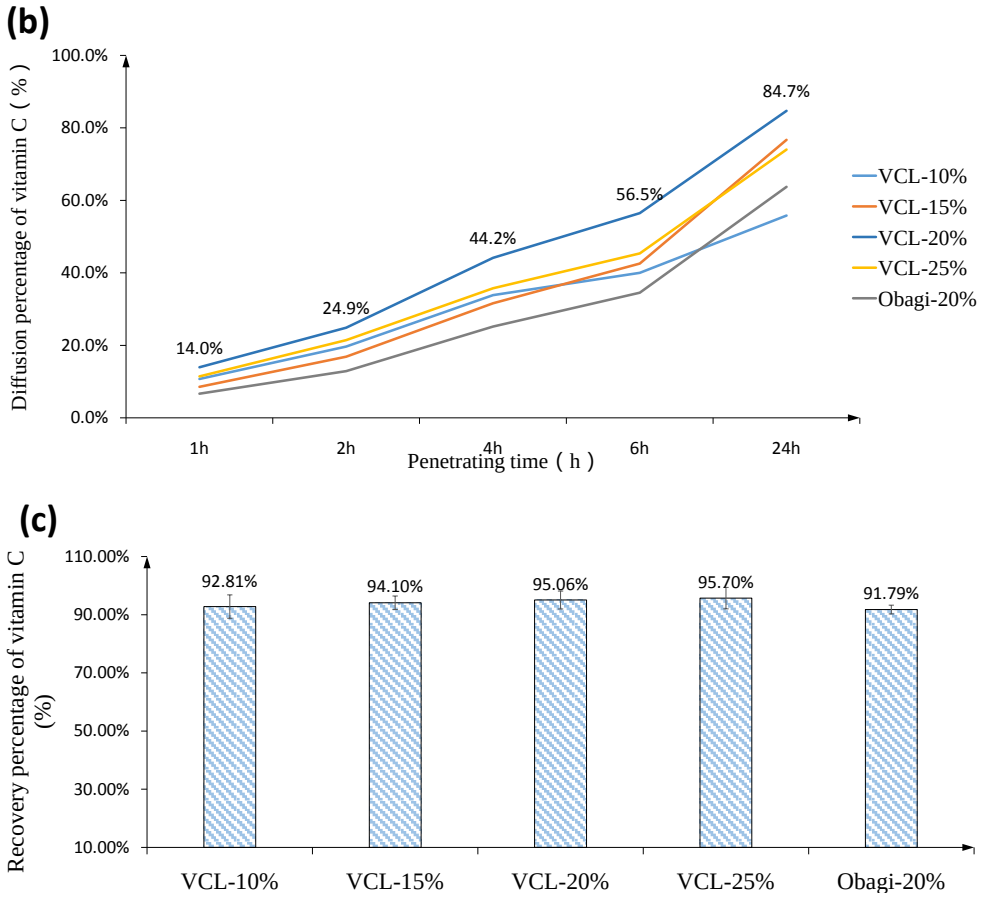


Figure 1. Transdermal penetration results of vitamin C lotion (VCL) on pig skin. (a) Cumulative permeability quantity of vitamin C, where Kp were the permeation coefficient. (b) Diffusion percentage of vitamin C. (c) Recovery rate of vitamin C. Besides, control group was the OBAGI C20 L-vitamin C essence (Obagi-20%).

While **Figure 1(b)** showed the highest diffusion percentage of VCL-20%, up to 84.71%, about 1.5 times of VCL-10% (55.79%). These results could be attributed to the complex transdermal mechanism of vitamin C. Concentration higher than 20% resulted in decreased permeation percentage for unknown reason [8].

Figure 1(c) showed the recovery percentage of vitamin C in tests, which of all the test samples were higher than 90%. Only a little vitamin C was lost, suggesting that the tests are credible.

Based on the results of diffusion percentage, 20% will be used as the optimal concentration of vitamin C. And the following tests will be performed with 20% vitamin C in lotion.

3.2. Eye Irritation Test *in Vitro*

Eye irritation was evaluated by cell viability of Rabbit corneal epithelial cells (RCE) [9]. As shown in **Figure 2**, there was a significant dose-dependent decrease in cell viability of RCE treated for 48 h with VCL. Even if the concentration of VCL is up to 10%, the cell viability of RCE was 94%, higher than 85%. These results indicated that there was no eye irritation of VCL.

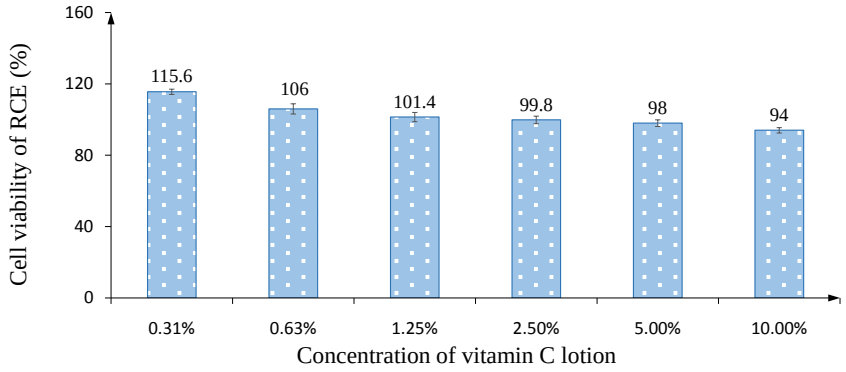


Figure 2. Cell viability of Rabbit corneal epithelial cultures after treated with vitamin C lotion.

3.3. Clinical Occlusive Patch Test

A clinical occlusive patch test was applied to evaluate the irritation of VCL. The values of visual scoring method are shown in **Table 3**, which is assessed by the doctor according to the safety technical specification for cosmetics (2015 Edition). Obviously, no erythema, no dryness and no edemas were observed during testing period of 0.5 h, 24 h and 48 h. The occlusive patch test results of all samples were negative, indicating that VCL will not cause skin allergic reaction.

3.4. VCL Prevents Melanin Synthesis *in Vitro*

Tyrosinase is the fundamental rate-limiting enzyme of melanin formation [10]. In this study, the effect of vitamin C lotion on melanin synthesis was analyzed by mice melanocytes culture. **Figure 3(a)** represented the prophylactic effects of VCL on tyrosinase activity. The tyrosinase activity inhibition rates were 9.10% and 12.50%, respectively, for 1 and 10 mg/ml compared to blank control.

Two concentrations of VCL could significantly decrease melanin synthesis, highlighting the concentration of 10 mg/ml. Most notably, the effect of VCL on tyrosinase was much lower than that of kojic acid, but its inhibition on melanin synthesis is stronger than that of kojic acid. These results suggested that inhibition of VCL on tyrosinase is not a unique path to affect melanin synthesis [11]. We reasonably speculate that VCL may affect melanin synthesis through other mechanisms, such as scavenging free radicals, reducing melanin and so on [12].

3.5. Improvement of Skin Color

Skin is the most perceivable indicator in the process of aging, with visible changes in the structure and function of the integument, such as pigmentation, sagging and wrinkle [13]. S. Venkatesh *et al.* [14] reported that East Asian populations are predisposed to hyperpigmentation but delayed wrinkle formation. As women of all ages are more likely to show skin aging than men, our subjects were all Chinese women. The skin color was evaluated at first through skin gloss and individual type angle (ITA˚), as shown by **Figure 4**.

Compared with initial state, the application of VCL for 1 day and 28 days increased the skin gloss by 31.58% and 10.53%, evidently higher than the control group ($p < 0.05$). Thus, we can infer that VCL could significantly enhance the skin gloss. Notably, skin gloss improvement percentage didn't increase with usage time, which may be attributed to multiple factors, such as the season, pressure and lifestyle.

Table 3. Visual assessment scores of clinic occlusive patch test.

Sample	Number of subjects	Time of duration (h)	Number of subjects in each visual assessment score				
			0 grading	1 grading	2 grading	3 grading	4 grading
Vitamin C lotion (VCL)	34	0.5	34	0	0	0	0
		24	34	0	0	0	0
		48	34	0	0	0	0
Blank control group	34	0.5	34	0	0	0	0
		24	34	0	0	0	0
		48	34	0	0	0	0

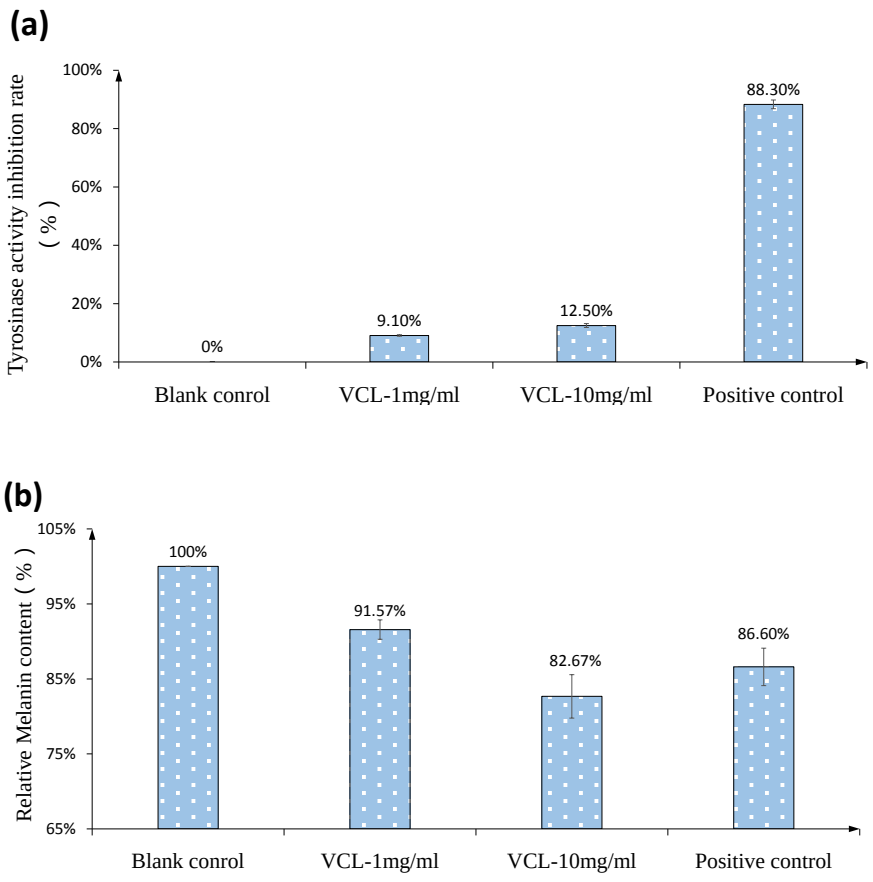


Figure 3. Effect of vitamin C lotion (VCL) on melanin synthesis in mice melanocytes cultures. (a) Tyrosinase activity inhibition rate. (b) Relative Melanin content. Positive control is Kojic acid of 0.5 mg/ml and blank control is normal saline.

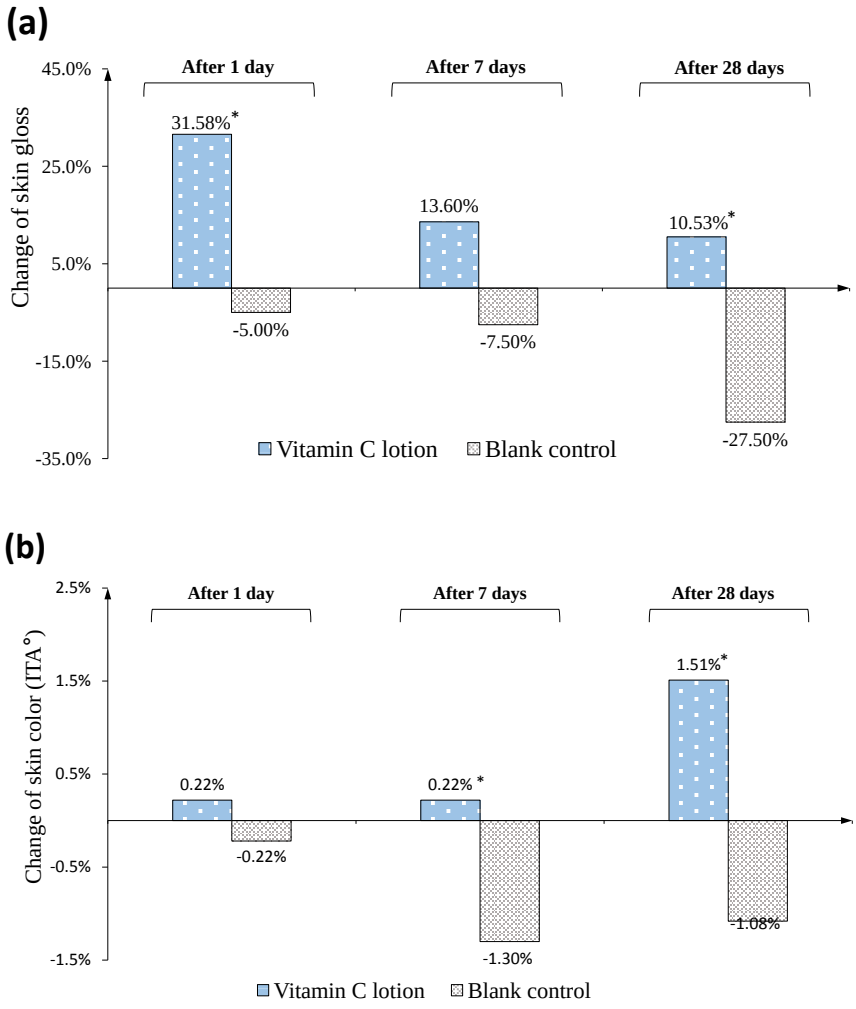


Figure 4. Changes of skin gloss and skin color (ITA˚). (a) Skin gloss change of subjects during the test. (b) Skin color (ITA˚) change of subjects during the test. *$p < 0.05$ vs. control group.

There was a positive correlation between individual type angle and skin gloss. As shown in **Figure 4(b)**, the ITA value of subjects increased by 0.22% ($p < 0.05$) and 1.51% ($p < 0.05$) respectively after using VCL for 7 days and 28 days, significantly higher than that of blank control. These results were consistent with the skin gloss test results, suggesting that vitamin C could effectively improve the ITA value and brighten skin in vehicle of lotion.

3.6. Improvement of Skin Elasticity and Firmness

The loss of structural elastin due to aging results in the skin's inability to stretch and recoil (decrease in elasticity) and manifests as loss of skin firmness and sagging [15]. This study used skin elasticity and firmness to evaluate facial state as two important indexes.

Figure 5(a) showed the skin elasticity change of subjects. After using VCL for 28 days, the skin elasticity of subjects increased by 3.86%, significantly higher

than initial value and blank control group ($p < 0.05$). Meanwhile, the blank control group had no significant effect on skin elasticity ($p > 0.05$). In summary, VCL could significantly improve skin elasticity of subjects after being used on the facial skin for 28 days.

The F4 parameter is negatively correlated with skin firmness. The smaller F4 value means better firmness, as represented in Figure 5(b). After 7 days and 28 days of use, the F4 value of the experimental group decreased obviously by 6.90% and 9.20% respectively, which were significantly better than initial value and the blank control group. Those results indicated that the application of VCL could significantly improve the facial firmness of subjects ($p < 0.05$).

3.7. Improvement of Facial Wrinkles

Wrinkles are a significant sign of aging skin, so it could be used to evaluate the anti-aging effect of active compounds as an important indicator. Figure 6 showed the skin wrinkle area and SA value of subjects during the test. After using vitamin C lotion for 7 days and 28 days on the facial skin, the skin wrinkle area of subjects significantly decreased by 8.79% ($p < 0.05$) and 12.27% ($p < 0.05$), and

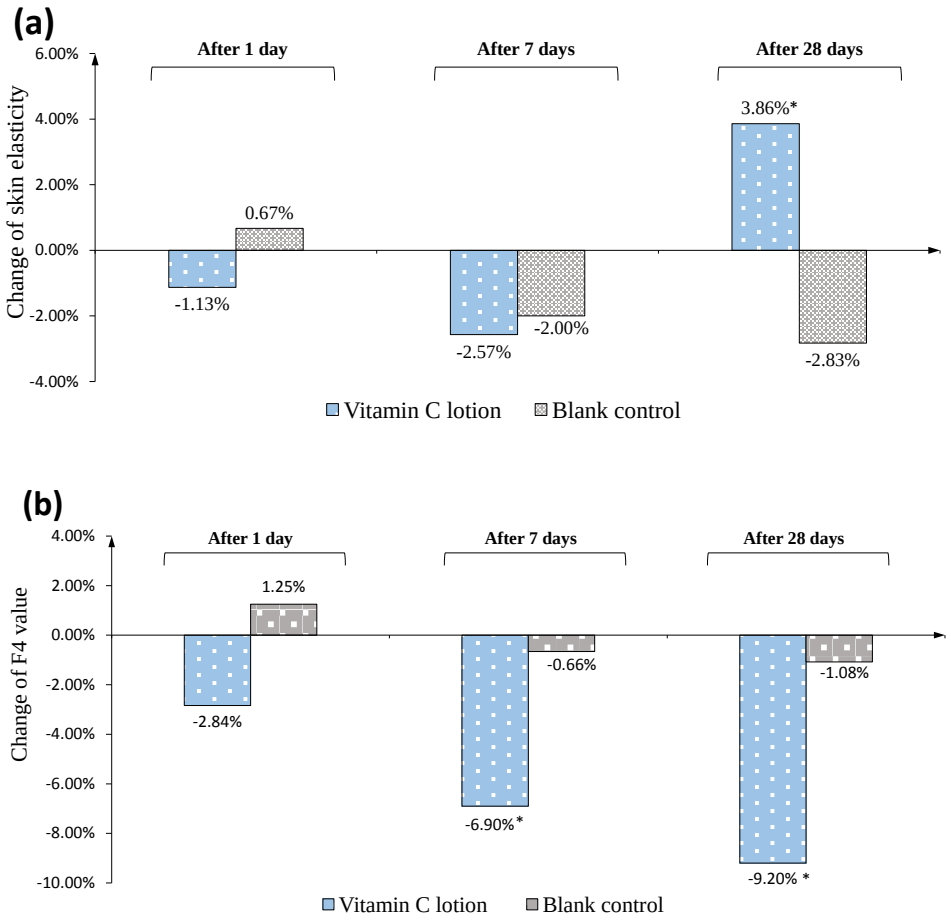


Figure 5. Changes of skin mechanical properties. (a) Skin elasticity (b) F4 value. *$p < 0.05$ vs. control group.

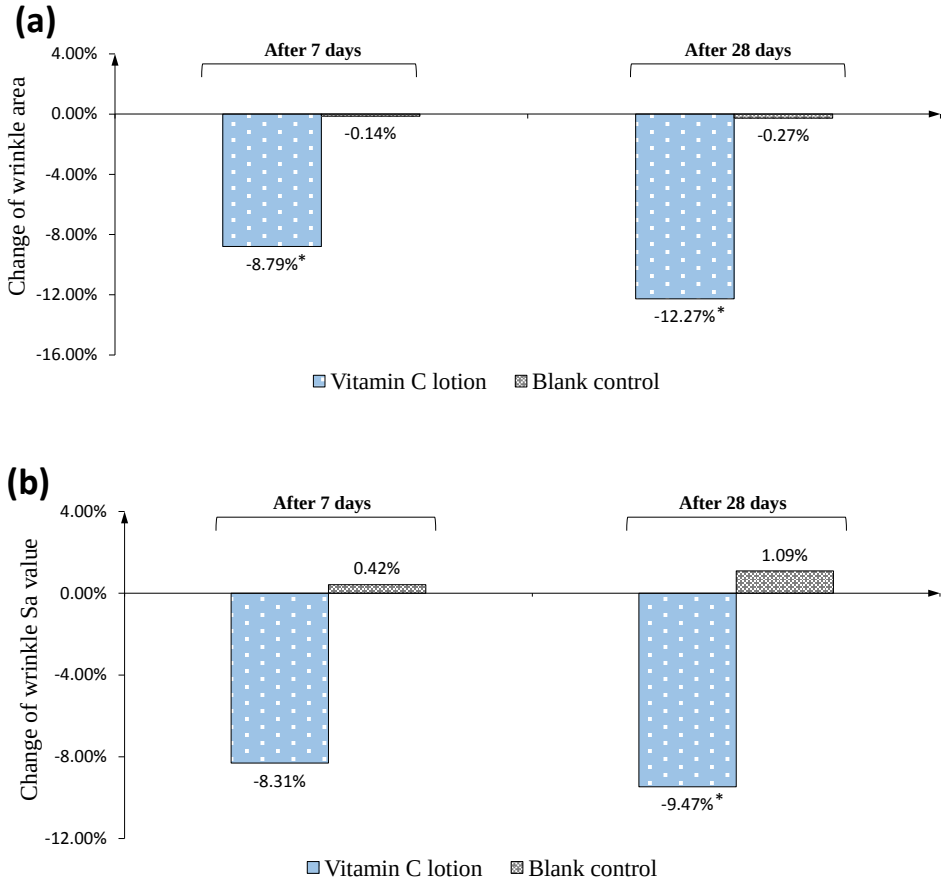


Figure 6. Changes of skin wrinkle area and wrinkle Sa value after using test products. (a) Skin wrinkle area of subjects. (b) Wrinkle SA value of subjects. *$p < 0.05$ vs. control group.

the wrinkle SA value decreased by 8.31% and 9.47% ($p < 0.05$), respectively. By contrast, the subjects' wrinkles were not improved significantly after using the control group. Above all, these results implied that VCL could effectively decrease the area and depth of facial wrinkles, and improve the appearance of aging skin.

4. Discussion

A series of changes due to intrinsic and extrinsic reasons will occur in the process of aging, resulting in the change of skin appearance eventually. Scientists have found that collagen in the skin is closely related to gender, and the content of collagen in women's skin was significantly lower than that in men at all ages [16]. Therefore, women should pay more attention than men to anti-aging skin care.

According to photoaging theory, an important reason for aging is the imbalance of oxidation in the skin. The skin is exposed daily to ultraviolet radiation, and these stimuli are known to increase reactive oxygen species (ROS), aggravating skin aging [17]. High ROS levels have numerous toxic and harmful effects [18]. Vitamin C is one of the non-enzymatic antioxidants in the human body and has excellent antioxidant properties. Patricia K Farris [19] reported that the use of topically applied vitamin C could delay photoaging through eliminating

ROS. These studies may explain the anti-aging efficacy of vitamin C on women's skin, including skin whitening, elasticity enhancement and wrinkle reduction (**Figures 4-6**).

There is a direct relationship between skin color and content of epidermal melanin. Ultraviolet light and inflammation can increase tyrosinase activity and promote melanin production. Tie-Chi Lei [20] found that vitamin C could suppress tyrosinase activity through cytoplasmic acidification. Thus, vitamin C could efficiently reduce the melanin synthesis to lightening the skin of subjects, which consists with the results in **Figure 4**.

The skin elasticity and wrinkles are affected by the content of collagen in the dermis. Skin collagen decreased with age and was less in the females at all ages [21]. Consequently, enhancement of collagen synthesis plays a vital role in the anti-aging process. PA Jones [22] has reported that the appearance of insoluble collagen in the extracellular matrices was completely dependent on the presence of vitamin C (ascorbic acid). **Figure 5** and **Figure 6** have shown the improvement of VCL on skin elasticity and wrinkles, which could be attributed to the insoluble collagen synthesis.

In addition, studies have reported that vitamin C can promote the differentiation of epidermal cells to ensure the integrity of the skin barrier [23]. To sum up, vitamin C can resist free radical scavenging, inhibit tyrosinase activity, help collagen synthesis and promote the integrity of epidermal barrier. We reasonably speculate that the application of VCL could efficiently improve the appearance of aging skin due to the potential efficacy of vitamin C, making the user's skin brighter, more elastic and fewer wrinkles.

5. Conclusion

In this study, vitamin C lotion was evaluated systematically *in vitro* and *in vivo*, including its transdermal penetration, safety and functional properties. At first, Franz cell system tests showed that vitamin C could effectively penetrate skin, and its transdermal efficiency was the highest of 84% with 20% vitamin C (mass fraction) in the lotion. Safety results showed that vitamin C lotion has low cytotoxicity to rabbit corneal epithelial cells, which was consistent with the results of occlusive patch test. In summary, the safety of vitamin C lotion was reliable according to the test results *in vivo* and *in vitro*. Besides, vitamin C lotion could obviously inhibit tyrosinase activity and reduce the amount of melanin *in vitro*, which has also been confirmed in following clinical tests. Unsurprisingly, vitamin C lotion also showed excellent functions of skin whitening and anti-aging on female subjects after being used for 28 days. Above all, we may confirm that this product could effectively brighten the skin color, improve skin elasticity and reduce facial wrinkles of subjects.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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